ZOOLOGICAL SCIENCE 6: 295-301 (1989)

The 5S RNA Binding Protein of *Artemia salina* Ribosomes: Identification and Immunological Comparison with That of Rat Liver

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ABSTRACT—The ribonucleoprotein complex between 5S RNA and its binding protein of *Artemia salina* ribosomes was released from 60S subunits with 25 mM EDTA. The protein component of the complex was found to contain a single ribosomal protein which seemed to correspond to rat liver ribosomal protein L5 in two-dimensional gel electrophoresis. We therefore designated the 5S RNA binding protein of *Artemia salina* as *Artemia* L5 (AL5) when compared with rat liver L5. The molecular weight of AL5 was about 34,000 in SDS-polyacrylamide gel electrophoresis and was similar to rat liver L5. The 5S RNA-AL5 complexes were injected into rabbits intradermally, and antisera against AL5 were obtained. Cross-reactivity of the anti-AL5 with rat liver 60S proteins was examined by immunoblotting and the anti-AL5 was found to cross-react with rat liver L5. This result indicated that *Artemia* and rat liver L5 share immunological determinants. These results suggest that 5S RNA binding proteins are probably conserved during the evolution of eukaryotic cells and may play a significant role in ribosomes.

INTRODUCTION

Ribonucleoprotein complexes between 5S RNAs and their binding proteins have been released from both prokaryotic and eukaryotic ribosomes [1–5]. The structure of 5S RNAs is well known, but the role of 5S RNA-protein complex is not yet known except for the functions of tRNA binding [6] and subunit association [7]. However, our studies on rat liver 5S RNA-protein complex have revealed that the protein moiety of the complex (rat liver L5) cross-linked to poly A^+ mRNA in the polysome and to globin mRNA in the 80S initiation complex by ultraviolet irradiation [8, 9], and cross-linked to proteins S4 and S25 by treatment of 80S ribosomes with 2 iminothiolane [10]. These results suggest that rat liver 5S RNA-L5 complex is located at the boundary between the large and the small subunits and interacts with mRNA.

Three ribosomal proteins (EL5, EL18, EL25) in *E. coli* and one each in yeast (YL3) and in rat liver (L5) are known to bind to 5S RNA [3–5]. In a study of the amino acid sequence of YL3 in yeast, Yaguchi *et al.* [11] indicated that a single large eukaryotic 5S RNA binding protein may have evolved through a fusion of genes for the multiple 5S RNA binding proteins in prokaryotes. This is an interesting suggestion concerning the evolution of ribosomal proteins.

In the present work we identified the 5S RNA binding protein of *Artemia salina* ribosomes and compared it to that of rat liver. Furthermore, the immunological homology between the proteins of *Artemia salina* and rat liver was examined by using the antibody against the 5S RNA binding protein of *Artemia* ribosomes.

Accepted April 26, 1988
Received May 27, 1988

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MATERIALS AND METHODS

Preparation of the 5S RNA-protein complex from Artemia ribosomes

Ribosomes were prepared as described previously [12] and large amount of 60S subunits were obtained by means of zonal centrifugation originally described by Sherton *et al.* [13]. About 1 g of ribosomal pellet was suspended in 100 ml of a high-salt buffer containing 20 mM Tris/HCl (pH 7.8), 0.5 M KCl, 3 mM $MgCl_2$, 20 mM 2-mercaptoethanol and 9% sucrose and sedimented through a 13–37% hyperbolic sucrose density gradient in the high-salt buffer at 13,500 rpm for 18 hr in a Hitachi RPZ 35T rotor at 18°C. The resulting profile of absorbance at 260 nm is shown in Figure 1. Fractions of 60S subunits were collected and pelleted by centrifugation at 130,000×*g* for 15 hr.

The 5S RNA-protein complex was prepared from 60S subunits by suspending the pellet in 25 mM EDTA followed by centrifugation according to Terao *et al.* [5]. About 1.6 mg of ribonucleoprotein was obtained from 1 g of *Artemia* ribosomes.

Electrophoretic analysis of the 5S RNA-protein complex and its binding protein

Disc gel electrophoresis of the 5S RNA-protein complex was carried out as described previously [14]. Two-dimensional gel electrophoresis of the 5S RNA binding protein was performed in an acidic-SDS gel system according to the method of Madjar *et al.* [15] with slight modification. The protein sample was dissolved in buffer consisting of 8 M urea, 1% 2-mercaptoethanol and 10 mM bis-Tris/acetic acid (pH 4.0) and applied to disc gel containing 4% acrylamide, 0.1% N, N'-tetramethylethylendiamine (TEMED), 8 M urea

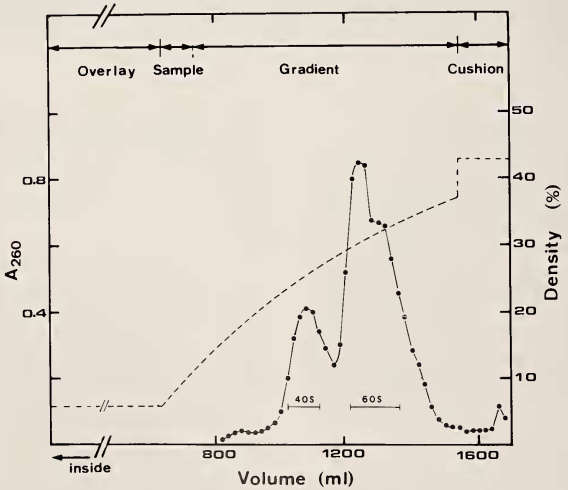

FIG. 1. Sedimentation profile of ribosomal subunits of *Artemia salina* in a zonal rotor. Ribosomal subunits (about 1 g) were separated by centrifugation for 18 hr at 13,500 rpm in a Hitachi RPZ 35T rotor at 18°C using a 13 to 37% hyperbolic sucrose density gradient in the high salt buffer described in MATERIALS AND METHODS. Fractions of 60S subunits (150 ml) were collected and about 400 mg of 60S subunits were recovered. The interrupted lines indicate densities of sucrose; 5.8% as to "overlay", 5.8–13% linear gradient as to "sample", 43% as to "cushion".

and 40 mM bis-Tris/acetic acid (pH 5.0). The upper and lower electrode buffers, which contained 10 mM bis-Tris, were adjusted to pH 4.0 and pH 6.0 with acetic acid, respectively. Electrophoresis in the first dimension was run at 150 V for 6 hr. After the gel was incubated in dialyzing buffer consisting of 6 M urea, 1% SDS and 40 mM bis-Tris/acetic acid (pH 6.0), the second dimensional SDS-polyacrylamide gel electrophoresis was carried out in the manner described previously [12].

Preparation of antibodies against the 5S RNA-protein complex

Antisera against *Artemia* 5S RNA-protein complexes were raised in rabbits by injecting the complexes intradermally with complete Freund's adjuvant (1:1 vol). Rabbits were injected with 0.3–0.5 mg of the complexes every two weeks and the total amount of antigen injected into one animal over the whole immunization period was 0.9 mg of protein. Immunoglobulins were precipitated from antisera at 40% saturation with ammonium sulfate and the IgG fractions were purified by DEAE-cellulose column chromatography. ^{125}I-labeled IgG was prepared with the Bolton-Hunter ^{125}I reagent (2,000 Ci/mmol) from New England Nuclear.

Immunoblotting procedures

Sixty S ribosomal proteins separated by two-dimensional acidic-SDS acrylamide gel electrophoresis were transferred to a nitrocellulose membrane sheet essentially according to the method of Towbin *et al.* [16]. Blotting was performed under a constant 200 mA current for 20 hr at room temperature in buffer containing 25 mM Tris, 192 mM glycine and 20% methanol at pH 8.2. The sheet was then soaked in 2% BSA (bovine serum albumin) in PBS (0.15 M NaCl and 10 mM sodium phosphate buffer, pH 7.2) for 2 hr. ^{125}I-labeled IgG (5×10^5 cpm) was added to 5 ml of 2% BSA in PBS and incubated with the sheet for 4 hr at room temperature. The membrane sheet was washed in 2% BSA in PBS for 5 min and rinsed twice with PBS. The sheet was air dried and autoradiographed with Fuji X-ray film for 18–48 hr.

RESULTS

Analysis of the 5S RNA-protein complex by disc gel electrophoresis

As previously observed with yeast and rat liver ribosomes [4, 5], the 5S RNA-protein complex was released from *Artemia* 60S ribosomes with 25 mM EDTA. The split-7S fraction released from EDTA-treated *Artemia* 60S ribosomes was analyzed by disc gel electrophoresis and compared with that from rat liver (Fig. 2). The split-7S fraction of *Artemia salina* contained two components and one of them was shown to be a ribonucleoprotein complex by staining the gel with Coomassie brilliant blue. The two components corresponded to rat liver 5S RNA-protein complex and free 5S RNA, respectively. SDS gel electrophoresis of the ribonucleoprotein complex showed that the complex consisted of two components: 5S ribosomal

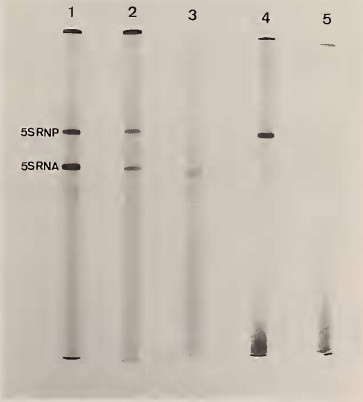

FIG. 2. Disc gel electrophoresis of the split-7S fraction released from *Artemia* and rat liver 60S ribosomes with 25 mM EDTA. Gels were stained with Azur B (lanes 1–3) or with Coomassie brilliant blue (lanes 4 and 5). Lanes 1 and 4; the split-7S fraction from *Artemia* 60S ribosomes, lanes 2 and 5; those from rat liver 60S ribosomes, lane 3; rat liver 5S RNA. "5S RNP" and "5S RNA" indicated the positions of rat liver 5S RNA-protein complex and 5S RNA, respectively.

RNA and a single ribosomal protein (data not shown). Thus, similarly to rat liver and yeast ribosomes, the 5S RNA-protein complex is released from *Artemia* 60S ribosomes with 25 mM EDTA and may contain a single ribosomal protein.

Two dimensional gel electrophoresis of the 5S RNA binding protein

Two dimensional gel electrophoresis was carried out to identify the 5S RNA binding protein of *Artemia* ribosomes. As shown in Figure 3, a single ribosomal protein spot is almost undetectable in the EDTA-treated 50S ribosomes but exists in the split-7S fraction. This protein seemed to correspond to rat liver ribosomal protein L5 (5S RNA binding protein) on the two dimensional gel slab, so we designated this protein *Artemia* L5 (AL5). The molecular weight of this protein is found to be about 34,000 by SDS gel electrophoresis, and this is the same as that of rat L5.

Characterization of the antibody against the 5S RNA-AL5 complex

In *E. coli* ribosomes, antibodies against each ribosomal protein have proved very useful in studying their functions [17, 18]. On the other hand, few antibodies against ribosomal proteins were able to be obtained in eukaryotic ribosomes. Therefore, we tried to obtain an antibody against *Artemia* L5 and intended to examine the function of the 5S RNA-protein complex by using the antibody. The 5S RNA-AL5 complexes were injected into rabbits intradermally and the antisera were obtained. In a series of preliminary experiments, the antisera were assayed for the formation of a precipitin line by Ouchterlony double diffusion with the 5S RNA-AL5 complex, the protein AL5, total proteins from *Artemia* 60S ribosomes or 40S ribosomes, and RNAs from *Artemia* 60S ribosomes. In the results the antisera cross-reacted with the 5S RNA-AL5 complex, the protein AL5 and total proteins from 60S ribosomes, but not with the others (data not shown). These results indicate that this antisera are specific for the protein moiety of the 5S RNA-protein complex.

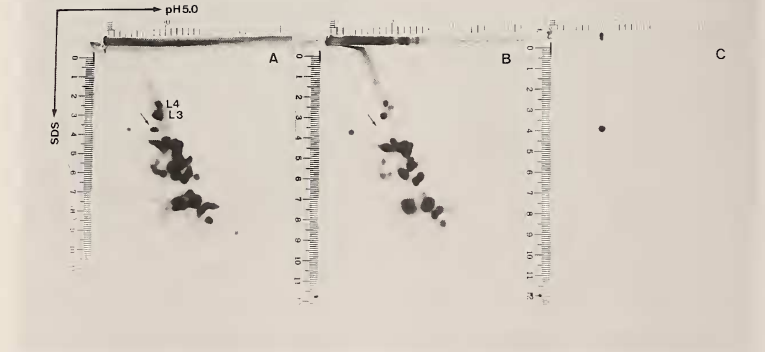

FIG. 3. Analysis of the protein of the split-7S fraction by two-dimensional gel electrophoresis. *Artemia* 60S ribosomal proteins (A), the core-50S ribosomal proteins derived from *Artemia* 60S ribosomes with 25 mM EDTA treatment (B) and protein of the split-7S fraction released from *Artemia* 60S ribosomes (C). Electrophoresis was run at pH 5.0 in the first dimension and in the presence of SDS in the second. The arrow shows the *Artemia* L5.

To show the specificity of the antibody, immunoblotting was carried out. Sixty S proteins of *Artemia* ribosomes separated by two dimensional gel electrophoresis were transferred to a nitrocellulose sheet and ^{125}I-labeled IgG from the antisera was treated. As shown in Figure 4, this antibody cross-reacted with AL5 only.

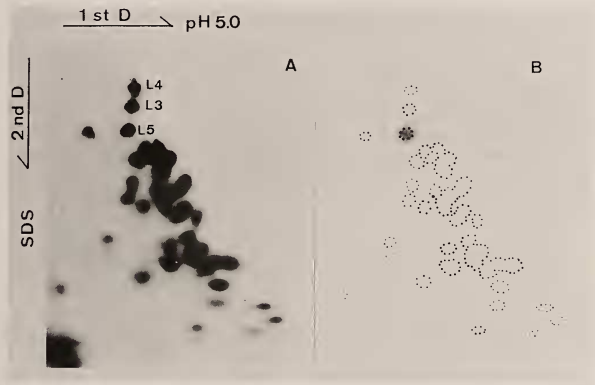

FIG. 4. Two-dimensional gel electrophoretogram of *Artemia* 60S ribosomal proteins (A) and autoradiogram by immunoblotting (B). Sixty S ribosomal proteins separated by two-dimensional acidic-SDS acrylamide gel electrophoresis were transferred to a nitrocellulose membrane sheet and the sheet was treated with ^{125}I-labeled IgG (5×10^5 cpm) and then autoradiographed.

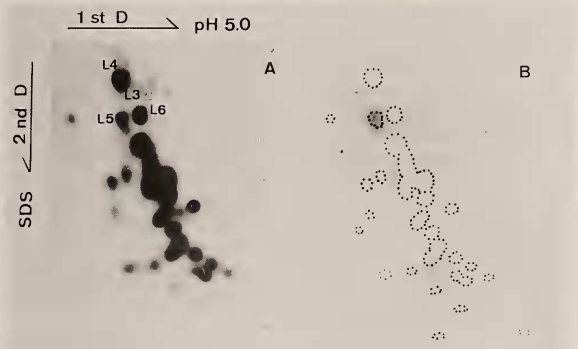

FIG. 5. Cross-reactivity of anti-AL5 with rat liver 60S ribosomal proteins by immunoblotting. Rat liver 60S ribosomal proteins were separated by acidic-SDS two-dimensional gel electrophoresis (A) and transferred to a nitrocellulose membrane sheet. The sheet was treated with ^{125}I-labeled anti-AL5 IgG (5×10^5 cpm) and then subjected to autoradiography (B).

Immunological homology between Artemia and rat liver L5

The cross-reactivity of anti-AL5 with rat liver 60S proteins was then examined by immunoblotting as described above. As shown in Figure 5, anti-AL5 was found to cross-react with a single protein of rat liver 60S proteins. This protein was L5, which was the 5S RNA binding protein of rat liver ribosomes. This indicates that *Artemia* and rat liver L5 share immunological determinants. Although correspondence between individual 60S proteins of *Artemia* and rat liver was very difficult to find [12], the protein L5 is shown to be highly conserved between the two species.

DISCUSSION

The 5S RNA binding protein of *Artemia* ribosomes (AL5) was a basic protein with a molecular weight of about 34,000, apparently the third largest protein component in the 60S ribosomal subunit. It was located in the same position as rat liver L5 on the two-dimensional gels. In our previous study on *Artemia* ribosomal proteins by two dimensional gel electrophoresis [12], we were unable to exactly detect AL5 which corresponded to rat liver L5 on the two dimensional gel slab. We detected only a faint spot of AL5 on the two dimensional gel slab. The basic-acidic and the basic-SDS gel systems were formerly used, where the first dimensional electrophoresis was run at pH 8.6. But in the present work, the protein corresponding to rat liver L5 was detected with the acidic-SDS gel system. Fabijanski and Pellegrini pointed out that many ribosomal proteins were only slihgtly soluble at pH 8.6 without thiol reducing agents [19]. Therefore, they used the acidic gel (pH 5.0) for the first dimension. In yeast ribosomes, the 5S RNA binding protein was largely insoluble in the first dimensional buffer at pH 8.6 and most of the protein remained trapped at the origin [4]. Consequently, AL5 also seemed to be insoluble and unable to be detected in the previous gel systems as in the case of yeast ribosomes.

In *E. coli* ribosomes, antibodies to each ribosomal protein were very useful in elucidating their functions [17, 18], but in the case of eukaryotic ribosomes few antibodies against each ribosomal protein were available because of the difficulty of obtaining sufficient pure proteins for immunization. We succeeded in obtaining the antibody against AL5 in the present work, so the antibody would become an effective means to clarify the function of the 5S RNA-protein complex. At first we showed that there exists an immunological homology between *Artemia* and rat liver L5 by immunoblotting. As to the amino acid sequences of the 5S RNA binding proteins, Tamura *et al.* [20] showed that 18 residues of amino acids in the N-terminal region and 28 in the C-terminal of rat liver L5 were identical with those of yeast YL3 (5S RNA binding protein). Both their results and ours indicate that a 5S RNA binding protein is probably conserved during the evolution of yeast to mammalian cells. After analyzing the amino acid sequences of YL3, Yaguchi *et al.* [11] suggested that the single large eukaryotic 5S RNA binding protein might have evolved through a fusion of genes for the multiple 5S RNA binding proteins in prokaryotes. Analysis of the amino acid sequences of AL5 is required to clarify this point of view.

Fabijanski and Pellegrini [19] indicated that rat liver L5 was located at or near the P site in affinity labeling experiments. Uchiumi *et al.* [10] showed that rat liver L5 cross-linked to S4 and S25 at the interface between the small and large subunits. Rat liver L5 may be located near the initiation region of 40S subunits in the 80S couples since S4 and S25 appeared to cross-link to eIF-3 [21, 22]. Concerning the location of *Artemia* L5 on ribosomes, the ability of the anti-AL5 to bind to the ribosomes or their subunits was examined and it was proved that the anti-AL5 did not react to 80S ribosomes but to 60S subunits [23]. Consequently, *Artemia* L5 is probably located at the interface between the two subunits as rat liver L5 and may play a significant role in protein synthesis at or near the initiation region of ribosomes.

ACKNOWLEDGMENTS

We thank Mr. Y. Maeda for excellent technical assistance in the immunization of rabbits. We also thank Dr. N. Sato for encouragement and support during the course of this work.

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